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Effects of neurosteroids on epileptiform activity induced by picrotoxin and 4-aminopyridine in the rat hippocampal slice

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Abstract

The neurosteroids allopregnanolone (5α-pregnan-3α-ol-20-one; 5α,3α-P) and its 5β-epimer pregnanolone (5β,3α-P), and pregnenolone sulfate (PS) were examined for effects on spontaneous epileptiform discharges induced by 100 μM picrotoxin (PTX) and 55 μM 4-aminopyridine (4-AP) in the CA3 region of the rat hippocampal slice. At a concentration of 10 μM, 5α,3α-P partially reduced PTX-induced bursting and at 30 and 90 μM completely suppressed bursting. In contrast, 100 μM 5β,3α-P failed to alter the discharge frequency. 5α,3α-P depressed 4-AP-induced bursting with similar potency as in the PTX model; 100 μM 5β,3α-P was also partially effective. In the 4-AP model, 5α,3α-P inhibited both the more frequent predominantly positive-going potentials as well as the less frequent negative-going potentials that may be generated by synchronous GABAergic interneuron firing. PS enhanced the PTX bursting frequency and, in the 4-AP model, increased the frequency of negative potentials but did not alter the frequency of positive potentials. By itself, PS did not induce bursting. The effects of the steroids in the in vitro seizure models largely correspond with their activities on $GABA_A$ receptors; suppression of discharges may occur as a result of direct activation of these receptors rather than modulation of GABA-mediated synaptic responses. PTX and 4-AP-induced bursting in the hippocampal slice are useful models for directly assessing neurosteroid effects on seizure susceptibility under conditions that eliminate the factor of brain bioavailability.

Keywords: Neurosteroid; Picrotoxin; 4-Aminopyridine; Hippocampal slice; Epilepsy

1. Introduction

Neurosteroids are endogenous steroids that influence the nervous system via cellular actions that do not involve steroid nuclear hormone receptors (Mellon and Griffin, 2002). The $GABA_A$ receptor is a key target for neurosteroids. Among the neurosteroids that act on $GABA_A$ receptors are certain 3α-hydroxy-pregnanes, such as allopregnanolone (5α-pregnan-3α-ol-20-one; 5α,3α-P), which are potent positive $GABA_A$ receptor modulators (Majewska et al., 1986; Puia et al., 1990; Majewska, 1992; Kokate et al., 1994; Lambert et al., 1995; Morrow et al., 1990; Gee et al., 1995). Other neurosteroids, including pregnenolone sulphate

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(PS), inhibit $GABA_A$ receptors and in addition act as positive modulators of NMDA receptors (Wu et al., 1991; Majewska, 1992; Bowlby, 1993; Irwin et al., 1994; Lambert et al., 1995). There are strict structural requirements for neurosteroid positive modulation of $GABA_A$ receptors (Gee et al., 1988; Purdy et al., 1990; Covey et al., 2001). In particular, pregnane steroid epimers with the 5β-configuration as in pregnanolone (5β-pregnan-3α-ol-20-one; 5β,3α-P) have reduced activity in potentiating $GABA_A$ receptors compared with their 5α-analogs (Morrow et al., 1990; Kokate et al., 1994).

In accordance with their actions on inhibitory synaptic transmission, $GABA_A$ receptor positive modulatory neurosteroids exhibit anticonvulsant activity in diverse animal seizure models (Belelli et al., 1989; Kokate et al., 1994, 1996; Leśkiewicz et al., 1997; Schwartz-Giblin et al., 1989; Członkowska et al., 2000; Frye et al., 2000). In contrast, PS has proconvulsant activity in animals, largely as a result of its $GABA_A$ receptor blocking activity which would reduce inhibitory synaptic transmission and possibly also because of its effects on NMDA receptors which would promote excitatory transmission (Maione et al., 1992; Reddy and Kulkarni, 1998; Kokate et al., 1999). Although there is extensive information on the activity of neurosteroids in animal seizure models, neurosteroids have not been shown to influence epileptiform activity in in vitro epilepsy models. Such in vitro models would be useful for defining neurosteroid effects on seizure susceptibility in the absence of confounding factors such as differences in absorption, metabolism, and brain accessibility. Therefore, in the present study, we sought to determine whether anticonvulsant and proconvulsant neurosteroids influence epileptiform bursting induced by two chemoconvulsants picrotoxin (PTX) and 4-aminopyridine (4-AP) in the in vitro hippocampal slice.

2. Methods

2.1. Preparation of hippocampal slices

All animal procedures were carried out in strict compliance with the NIH Guide for the Care and Use of Laboratory Animals under a protocol approved by the National Institutes of Neurological Disorders and Stroke Animal Care and Use Committee. Adult male rats weighing 120–180 g were decapitated under brief carbon dioxide narcosis. The brains were rapidly removed and immediately submerged in an ice-cold cutting solution. Transverse 500 μm thick hippocampal slices were prepared with a Vibratome and equilibrated before recording for at least 1 h in artificial cerebrospinal fluid (ACSF) bubbled with carbogen gas (95% O_2/5% CO_2) at 32–33 °C. The composition of the ACSF (in mM) was: 130 NaCl; 26 $NaHCO_3$; 3 KCl; 1.25 NaH_2PO_4; 2 $CaCl_2$; 1 $MgCl_2$; 10 D-glucose, pH 7.4. The cutting solution was similar to ACSF except that NaCl was replaced with isosmotic sucrose.

2.2. Electrophysiological recording and data analysis

After equilibration, the slices were transferred to an interface-type recording chamber that was continuously gravity perfused during the 1–2 h of the experiment with warmed (33 °C) ACSF at a flow rate of 2.5–3 ml/min. The surface of the slice was exposed to a gentle stream of humidified carbogen gas. Extracellular field recordings were carried out in the CA3 pyramidal layer with glass micropipettes (2–10 MΩ) filled with ACSF. The location of the recording electrode was optimized by maximizing the field potential amplitude evoked in response to a 100-ms test stimulus applied to the dentate gyrus mossy fibers. Amplified electrode signals were digitized and acquired using SCAN (Strathclyde Electrophysiology Software). Events were detected off-line with Mini Analysis Program (Synaptosoft, Decatur, GA). The parameters were empirically set to detect a maximum number of population responses that were clearly discernible from the background noise. The amplitude threshold was always ±0.2 mV, which is 10-fold greater than the typical background noise level of 0.02 mV. The event rate during the course of each experiment is expressed by plotting the frequency of events in successive 10-min intervals against the time of the recording. Comparisons between treatments were carried out with analysis of variance and *t*-test.

2.3. Drug application

To evoke spontaneous epileptiform discharges, the slice perfusion solution was changed from ACSF to

ACSF supplemented with PTX or 4-AP. In some experiments, neurosteroids were also added to the perfusion solution at the time of switch to the PTX- or 4-AP-containing ACSF. In other experiments, 60 min was allowed to elapse after the switch to PTX or 4-AP before the neurosteroids were added. Neurosteroids were solubilized in dimethyl sulfoxide (DMSO) or 2-hydroxypropyl-β-cyclodextrin before adding to the ACSF; the final concentrations of these solubilizing agents never exceed 0.2–0.3%. The solubilizers by themselves at these concentrations had no apparent effect on the epileptiform discharges. All drugs and steroids were purchased from Sigma (St. Louis, MO).

3. Results

3.1. Picrotoxin model

Although field responses in the CA3 area were readily evoked by mossy fiber stimulation, spontaneous events >0.02 mV in amplitude were not observed during perfusion with ACSF alone (see Fig. 2A). Addition of PTX (100 μM) to the perfusion solution resulted in the appearance of spontaneous positive–negative events of overall amplitude 1.5–2 mV and duration 100–250 ms (Fig. 1A). These events initially appeared at a low rate in the first few minutes after the beginning

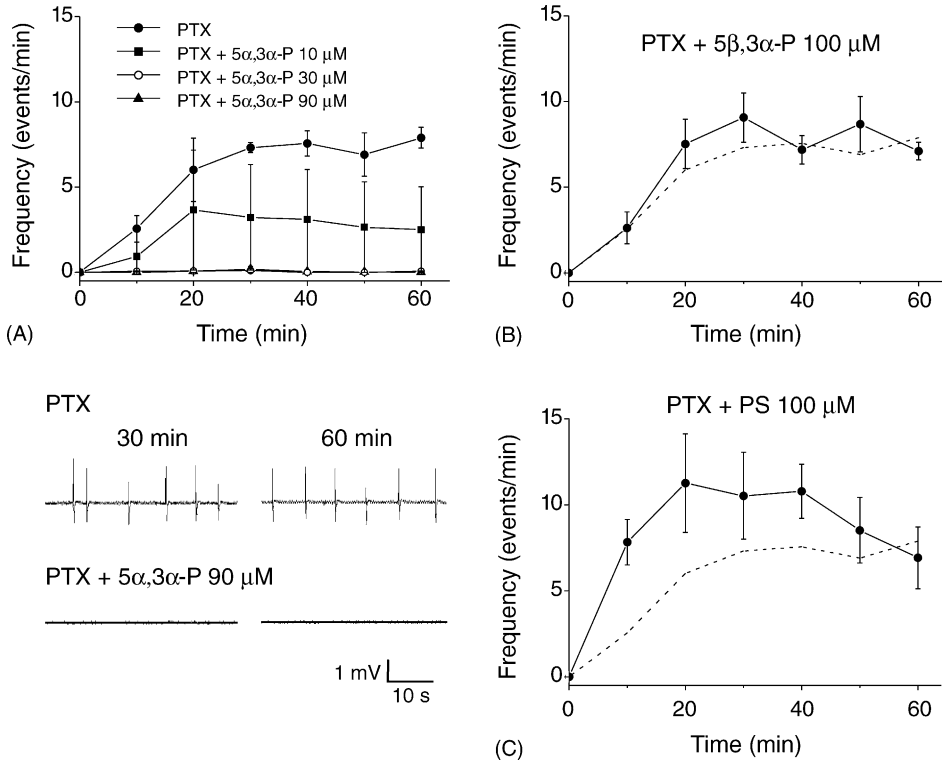

Fig. 1. Effects of neurosteroids on spontaneous epileptiform discharges induced by PTX. (A) Slices were perfused from the beginning of the experiment (time = 0 min) with 100 μM PTX. In some cases, 10, 30, or 90 μM 5α,3α-P was included along with the PTX. The frequency of spontaneous epileptiform discharges recorded extracellularly in hippocampal area CA3 was determined for successive 10 min epochs during the recording. All three concentrations of 5α,3α-P were associated with a significant reduction in event frequency ($P < 0.05$, 0.01, 0.01 for 10, 30, and 90 μM 5α,3α-P, respectively). Representative traces from experiments with PTX alone and 90 μM 5α,3α-P are shown at bottom. (B) In experiments with 100 μM 5β,3α-P, the mean event frequency values were not statistically different ($P > 0.05$) from those with PTX alone (dotted line plots control values from panel A). (C) Simultaneous perfusion with 100 μM PS was associated with an increased discharge frequency in comparison with PTX alone (dotted line) at all time points except 60 min. The mean discharge frequency value at 20 min was significantly different from control ($P < 0.05$). In all plots, each point represents the mean ± S.E.M. of event frequency values from four or five slices.

of the PTX perfusion and gradually increased in rate, reaching, within 20–30 min, a plateau frequency of approximately 6–8 events/min (maximum mean value, 8.9 ± 0.6 events/min) (Fig. 1A). In the continued presence of PTX, bursting at this rate continued for at least 60 min, at which time the recording was terminated.

5α,3α-P, 5β,3α-P, and PS were tested for their ability to influence the discharge frequency when added concurrently with PTX to the perfusion solution. In three of four experiments, 10 μM 5α,3α-P was associated with reduced bursting (maximal mean value, 3.7 ± 3.5 events/min at 60 min). At higher 5α,3α-P concentrations of 30 and 90 μM, PTX bursting was completely inhibited. The mean event frequencies in successive 10 min epochs following the onset of PTX perfusion are plotted in Fig. 1A along with representative traces of the extracellularly recorded field responses. In contrast to 5α,3α-P, 100 μM 5β,3α-P failed to affect the discharge rate (Fig. 1B).

Perfusion with 100 μM PS alone for up to 60 min did not induce epileptiform bursting (five slices; not shown). However, when 100 μM PS was included in the perfusion solution along with PTX, there was a potentiation in the discharge rate, with the frequency reaching a maximum value at 20 min of 11.3 ± 2.9 events/min compared with 6.0 ± 1.9 events/min in the absence of PS (Fig. 1C) ($P < 0.05$ at 20 min).

3.2. 4-Aminopyridine model

Perfusion with 55 and 75 μM 4-AP induced spontaneous bursting similar to that obtained with PTX except the plateau frequency was greater (maximal mean values, respectively, 34.0 ± 7.3 and 39.4 ±

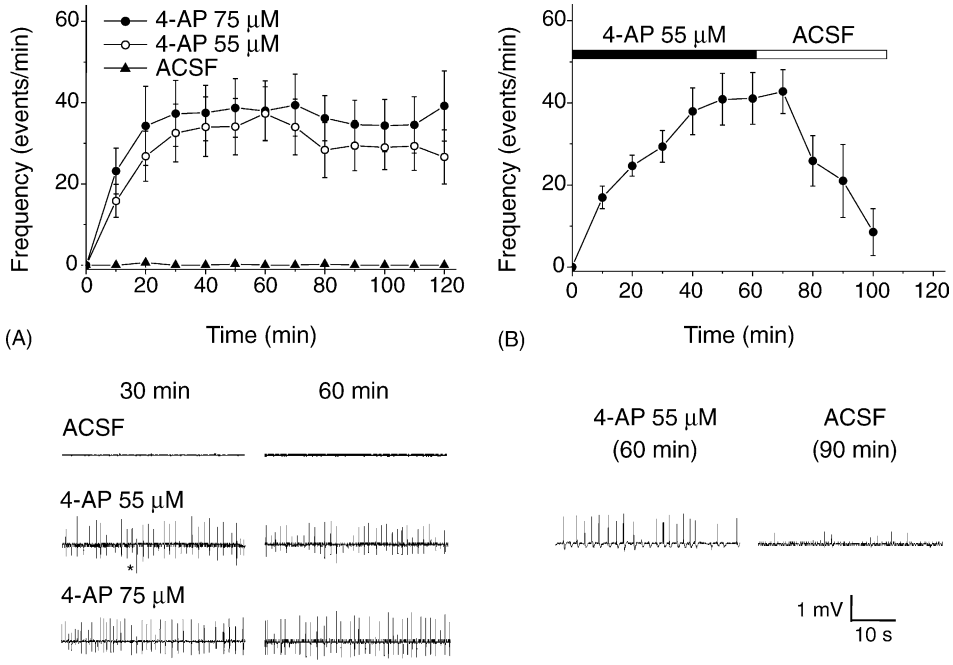

Fig. 2. Time course of the onset and recovery of spontaneous epileptiform discharges induced by 4-AP. The frequency of spontaneous epileptiform discharges recorded extracellularly in hippocampal area CA3 was determined for successive 10 min epochs during the recording. (A) The mean discharge frequency gradually increases, reaching a plateau 20–40 min after the addition of 55 or 75 μM 4-AP. There is no significant difference between the mean event frequencies obtained at the two 4-AP concentrations ($P > 0.1$). Representative traces from control (ACSF) experiments and with 55 and 75 μM 4-AP are shown at bottom. Asterisk marks typical predominantly negative-going potential. (B) Mean discharge frequency gradually rises to a plateau after 60-min exposure to 55 μM 4-AP (41.1 ± 6.3 events/min at 60 min) and slows to 8.5 ± 5.7 events/min at 100 min ($P < 0.05$) during the 40-min wash period. Representative traces from one experiment at 60 and 90 min are shown. Note the reduced burst amplitude at 90 min in the washout period. Each point represents the mean ± S.E.M. of event frequency values for eight slices, except for the control (ACSF) values in panel A, which represent three experiments.

7.7 events/min at 70 min) (Fig. 2). In addition, negative-going potentials were observed at lower frequency of 3–12 events/min (mean: 6.7±1.4 events/min), as previously reported (Avoli et al., 1996, 2002). Bursting at near the plateau rate persisted for as long as 4-AP was present in the medium (up to 2 h; Fig. 2A), but when 4-AP was removed from the perfusion medium, the discharge frequency gradually slowed over 40 min (Fig. 2B). Representative examples of the extracellularly recorded field responses in control- and 4-AP-treated slices are shown in the lower half of Fig. 2.

In slices exposed to 55 μM 4-AP for 60 min, addition of 90 μM 5α,3α-P to the perfusion solution caused a 93% decrease in bursting frequency within 40 min (Fig. 3A). This protective action of 5α,3α-P was also evident when the steroid was added together with 4-AP from the beginning of the perfusion (Fig. 3B). In this case, 30 and 90 μM 5α,3α-P nearly completely prevented the occurrence of epileptiform activity, whereas at 10 μM protection was observed in only three of four slices tested (Fig. 3B). In addition to suppressing the frequent predominantly positive-going potentials, 5α,3α-P also inhibited the negative-going potentials (Fig. 6A). In experiments with five slices exposed to 90 μM 5α,3α-P for 60 min and then switched to a perfusion solution containing 4-AP alone, bursting was not observed during the initial period of steroid exposure but bursting did develop along a similar time course as in steroid-naïve slices. However, the amplitude of the bursts was markedly reduced (<0.2 mV); events reaching the 0.2 mV amplitude threshold occurred at a rate of <3 events/s for as long as 60 min following initiation of the 4-AP perfusion. In contrast to 5α,3α-P, 100 μM 5β,3α-P failed to significantly affect 4-AP-induced bursting in slices exposed to 55 μM 4-AP, although there was a trend toward a reduction both in experiments where the steroid was added after bursting had been well established (Fig. 4A) and in experiments where 4-AP and the steroid were added together (Fig. 4B). Similarly, there was a non-significant trend toward

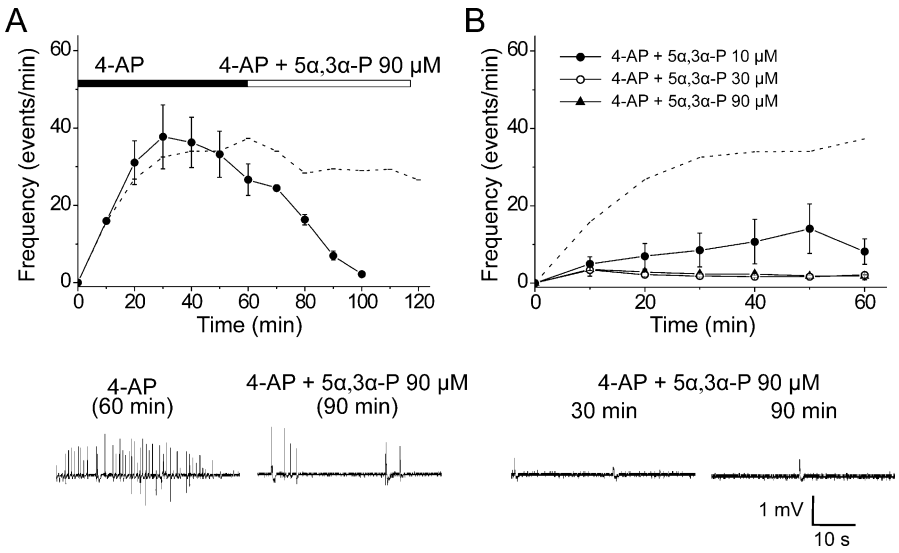

Fig. 3. Effects of 5α,3α-P on the frequency of spontaneous epileptiform discharges induced by 4-AP. (A) Following 60-min exposure to 55 μM 4-AP alone, 90 μM 5α,3α-P was added to the perfusion solution along with the 4-AP. The steroid caused a gradual reduction in the bursting frequency over the subsequent 40 min. Each point represents the mean ± S.E.M. of event frequency values for eight slices. The dashed line indicates mean control values for 55 μM 4-AP alone from the experiment of Fig. 2A; the difference between the control and 5α,3α-P data is significant by ANOVA ($P < 0.01$). Representative traces from one experiment at 60 and 90 min are shown. (B) Coapplication of 55 μM 4-AP and various concentrations of 5α,3α-P also results in significantly reduced bursting compared with control (dashed line) ($P < 0.05$, 0.01, 0.01 by ANOVA for 10, 30, and 90 μM 5α,3α-P; four, five, and five slices, respectively).

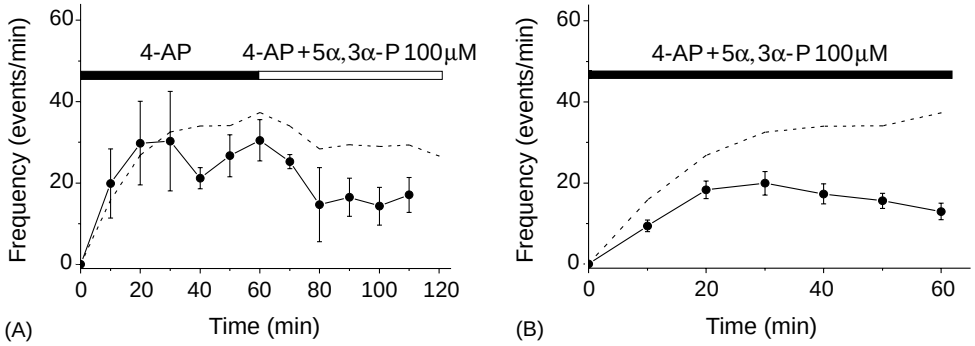

Fig. 4. Effects of 5β,3α-P on the frequency of spontaneous epileptiform discharges induced by 4-AP. (A) Following 60-min exposure to 55 μM 4-AP alone, 100 μM 5β,3α-P was added to the perfusion solution along with 4-AP. The steroid caused a small reduction in the mean burst frequency but this was not significantly different by ANOVA ($P > 0.05$) from the mean control values for 55 μM 4-AP alone from Fig. 2A (dashed line). Each point represents the mean ± S.E.M. of data from four experiments. (B) Coapplication of 55 μM 4-AP and 100 μM 5β,3α-P also tended to decrease the event frequency compared with control (dashed line), but this effect was not significant ($P > 0.05$; five slices) except at 60 min ($P < 0.05$).

inhibition of the negative potentials by 5β,3α-P (Fig. 6A).

In contrast to the situation with the PTX model, 10 or 100 μM PS did not significantly increase the discharge frequency of positive potentials when added to slices that had been exposed to 55 μM 4-AP for 60 min (Fig. 5A) and there was a non-significant trend toward reduced activity during the last 20–40 min of PS exposure when coapplied together with 55 μM 4-AP from the beginning of the recording (Fig. 5B). Bursting induced by 75 μM 4-AP was similarly unaffected by 10 and 100 μM PS when added to the perfusion solution after 60 min of 4-AP exposure (8 and 10 slices, respectively; data not shown). When slices were exposed to 100 μM PS for 1 h and subsequently perfused with 75 μM 4-AP in the absence of PS, the ensuing response to 4-AP was similar in magnitude and time course to that observed in control slices treated with 4-AP alone as shown in Fig. 2A (10 slices; data not shown). Although PS did not increase the frequency of 4-AP-induced positive potentials, it did markedly enhance the frequency of negative potentials (Fig. 6B).

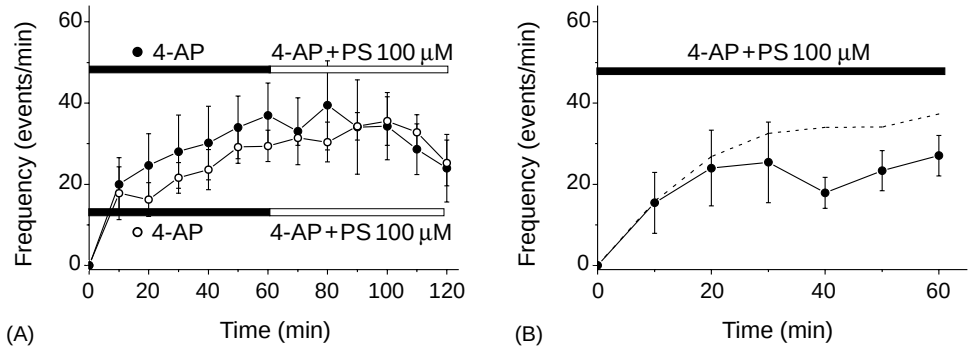

Fig. 5. Effect of PS on the frequency of spontaneous epileptiform discharges induced by 4-AP. (A) Following 60-min exposure to 55 μM 4-AP alone, 10 or 100 μM PS was added to the perfusion solution along with 4-AP. For both PS concentrations, the mean burst frequency values were not significantly different by ANOVA ($P > 0.1$) from the control values for 55 μM 4-AP alone from Fig. 2A. Each point represents the mean ± S.E.M. of data from four to five experiments. (B) Coapplication of 55 μM 4-AP and 100 μM PS tended to decrease the event frequency compared with control (dashed line), but this effect was not significant ($P > 0.05$; five slices).

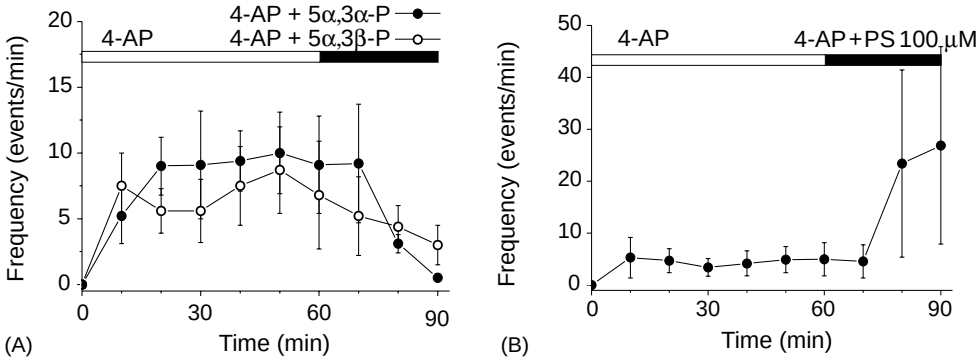

Fig. 6. Effect of 5α,3α-P, 5β,3α-P, and PS on the frequency of spontaneous negative potentials induced by 4-AP. (A) Following 60-min exposure to 55 μM 4-AP alone, 5α,3α-P and 5β,3α-P were added to the perfusion solution along with 4-AP (four slices each steroid). Both steroids caused a reduction in the mean frequency of negative potentials but the change was only significant for 5α,3α-P ($P < 0.05$). (B) Following 60-min exposure to 55 μM 4-AP alone, addition of PS to the perfusion solution along with 4-AP caused an increase in mean negative potential frequency in all three slices.

4. Discussion

The primary aim of this study was to test the modulatory activity of some neurosteroids on the epileptiform discharges induced by PTX and 4-AP in hippocampal slices. Our results show that 5α,3α-P, a steroid with well recognized anticonvulsant activity in diverse animal seizure models, produces a concentration-dependent suppression of epileptiform discharges in both models. In contrast, its 5β-epimer, 5β,3α-P at the concentration tested (100 μM) failed to produce a significant suppression in either model, although there was a trend toward reduced bursting in the 4-AP model. 5β,3α-P is approximately three-fold weaker than 5α,3α-P as a positive modulator of $GABA_A$ receptors (Gee et al., 1988; Kokate et al., 1994). In contrast, it is nearly equal in potency as an anticonvulsant in vivo (Kokate et al., 1994, 1996; Wieland et al., 1995; Gasior et al., 1997; Budziszewska et al., 1998). Similarly, 5β,3α-P is only slightly less potent than 5α,3α-P as a hypnotic and anesthetic (Atkinson et al., 1965; Gyermek, 1967; Gyermek et al., 1967; Norberg et al., 1987, 1999). Therefore, the in vitro antiepileptic activities of the pregane steroid epimers as assessed in the two models used here corresponds better with their in vitro activity on $GABA_A$ receptors than with their in vivo effects on seizures. This supports the concept that the anti-seizure activity of 5α,3α-P is related to its effects on $GABA_A$ receptors and suggests that other factors, such as differences in absorption, brain access or metabolism, could contribute to the relatively greater than expected in vivo activity of 5β,3α-P.

In the whole animal, 5α,3α-P confers protection against seizures induced by a broad range of chemoconvulsants, including the $GABA_A$ receptor antagonists pentylenetetrazol, PTX, and bicuculline, as well as kainate, NMDA, and pilocarpine (Belelli et al., 1989; Kokate et al., 1994, 1996; Leśkiewicz et al., 1997; Schwartz-Giblin et al., 1989; Członkowska et al., 2000; Frye et al., 2000). Therefore, it is not surprising that the neurosteroid is also effective against epileptiform discharges induced by PTX in vitro. However, in in vivo experiments, agents that potentiate GABAergic synaptic function have generally been found to be ineffective against 4-AP-induced seizures and lethality. Drugs in this category include vigabatrin, aminooxyacetic acid, tiagabine, nipecotic acid, NNC-711, muscimol, isoguvacine, and diazepam (Yamaguchi and Rogawski, 1992; Peña and Tapia, 2000), and also 11-deoxycorticosterone, a precursor of $GABA_A$ receptor modulatory neurosteroids (Reddy and Rogawski, 2002), as well as 5α,3α-P (P. Salazar and R. Tapia, unpublished data). In in vitro hippocampal slice recordings, GABAergic drugs including muscimol, phenobarbital, and pentobarbital reduce the frequency of the epileptiform discharges induced by 4-AP (Chesnut and Swann, 1990; Bruckner and Heinemann, 2000; Leniger et al., 2000; Fueta and Avoli, 1992). Thus, although our observation

that $GABA_A$ receptor modulating neurosteroids are highly effective against 4-AP-induced epileptiform activity in vitro is not expected from the lack of activity of GABAergic agents including 5α,3α-P against 4-AP-induced seizures in vivo, it is consistent with the prior brain slice studies.

In addition to their well recognized role as positive modulators of the natural agonist GABA, barbiturates including phenobarbital and pentobarbital can directly gate $GABA_A$ receptors in the absence of GABA (ffrench-Mullen et al., 1993; Rho et al., 1996). Neurosteroids have similar effects (Callachan et al., 1987; Kokate et al., 1994; Lambert et al., 1995; Reddy and Rogawski, 2002). The micromolar concentrations of 5α,3α-P that were found to be protective in the in vitro PTX and 4-AP seizure models were substantially greater than those that potentiate $GABA_A$ receptor responses in isolated cell systems, where effects may be seen at concentrations as low as 10–100 nM (Majewska et al., 1986; Gee et al., 1988; Puia et al., 1990; Woodward et al., 1992; Kokate et al., 1994; Park-Chung et al., 1999). They are also modestly greater than the concentrations (∼1 μM) which enhance and prolong the decay of $GABA_A$ receptor-mediated synaptic currents in brain slices (Brussaard et al., 1997; Haage and Johansson, 1999). However, they are in the same range as those that directly activate $GABA_A$ receptors in the absence of GABA (Callachan et al., 1987; Kokate et al., 1994; Lambert et al., 1995; Reddy and Rogawski, 2002). Therefore, it is interesting to speculate that the protective activity of 5α,3α-P in vitro may depend more upon its ability to activate $GABA_A$ receptors directly than to modulate GABA responses. In fact, the GABAergic agents that have previously been shown to affect 4-AP-induced bursting in vitro (muscimol, phenobarbital, and pentobarbital), all directly activate $GABA_A$ receptors (ffrench-Mullen et al., 1993; Rho et al., 1996). Moreover, there is some evidence that 5β,3α-P may be less efficacious as a direct agonist than 5α,3α-P (Turner and Simmonds, 1989), which is consistent with our observation that the 5β-steroid is not as active in the in vitro seizure models.

Why might direct activation of $GABA_A$ receptors be necessary to suppress bursting in the in vitro 4-AP model? 4-AP is a potassium channel blocker that indirectly enhances calcium entry into presynaptic nerve terminals and as a consequence powerfully stimulates neurotransmitter release as demonstrated by studies with synaptosomes in vitro (Thesleff, 1980; Tapia and Sitges, 1982; Tapia et al., 1985) and microdialysis in vivo (Morales-Villagrán and Tapia, 1996; Morales-Villagrán et al., 1996; Peña and Tapia, 1999, 2000; Medina-Ceja et al., 2000). As observed in the present study, 4-AP induces intense epileptiform activity in hippocampal slices (Voskuyl and Albus, 1985; Perrault and Avoli, 1991, 1992; Avoli et al., 1996; Southan and Owen, 1997; Peña et al., 2002). In addition, 4-AP is well recognized to be a powerful convulsant in animals and man (Spyker et al., 1980; Fragoso-Veloz and Tapia, 1992; Yamaguchi and Rogawski, 1992; Morales-Villagrán and Tapia, 1996; Morales-Villagrán et al., 1996). The convulsant activity of 4-AP seems to be largely due to enhancement of glutamatergic synaptic transmission. However, 4-AP is well recognized to increase neurotransmitter release at both excitatory and inhibitory synapses (Thesleff, 1980; Rutecki et al., 1987; Avoli et al., 2002). In fact, in the absence of glutamate-mediated synaptic transmission, 4-AP induces large potentials that are due to the synchronous firing of GABAergic interneurons (Aram et al., 1991; Avoli et al., 1996; Benardo, 1997). Since GABA interneurons are already highly active in the presence of 4-AP and the epileptiform activity appears to have escaped from inhibitory control, it is not surprising that potentiating phasic GABAergic inhibition does not protect against 4-AP-induced epileptiform activity and seizures. On the other hand, concentrations of neurosteroids that directly activate $GABA_A$ receptors would depress the circuit activity that underlies synchronous firing by inhibiting both the principal neurons responsible for excitatory transmission as well as the interneurons that generate the GABA-mediated inhibitory tone. In fact, in the present study, we found that 5α,3α-P suppressed both the frequent predominantly positive field potentials that are dependent upon glutamate-mediated synaptic transmission as well as the predominantly negative-going field potentials that are believed to reflect the synchronous activity of GABA neurons (Perrault and Avoli, 1992; Benardo, 1997). The inhibitory effect of 5α,3α-P on negative potentials supports the hypothesis that the protective action of the steroid in the in vitro seizure models results from direct activation of $GABA_A$ receptors and is not due to facilitation of synaptic GABAergic inhibition. Indeed, the reduction

in negative potentials implies that the firing activity of GABA neurons is markedly depressed (GABAergic synaptic tone is nearly absent) at a time when the steroid exerts strong inhibitory effects on epileptiform activity. (Proepileptic activity does not occur when GABAergic activity is depressed presumably because there is a corresponding inhibition of principal neuron activity.) In the in vivo situation, it may be difficult to achieve high enough concentrations of the steroids to produce the neuronal depression needed to block epileptiform activity, thus accounting for the failure of neurosteroids to protect against 4-AP seizures in the whole animal. However, in the in vitro slice, suppression of 4-AP-induced epileptiform discharges is readily achieved. Interestingly, high concentrations of 5α,3α-P have been reported to exert neuroprotective actions in hippocampal slices, primary neuronal cultures (Kajta et al., 1999; Frank and Sagratella, 2000), and human NT2 cells (Lockhart et al., 2002). These actions may similarly require direct activation of $GABA_A$ receptors.

In contrast to the anticonvulsant effects exerted by 5α,3α-P and related pregnane steroids, the sulfated pregnene steroid PS has proconvulsant activity in animals (Maione et al., 1992; Reddy and Kulkarni, 1998; Kokate et al., 1999). PS is well recognized to act as a negative allosteric modulator of $GABA_A$ receptors as demonstrated in studies of [^{35}S]*t*-butyl-bicylophosphorothioate binding, GABA agonist-activated Cl^- uptake in synaptoneurosomes, voltage clamp studies of native $GABA_A$ receptors in neurons and recombinant $GABA_A$ receptors expressed in *Xenopus* oocytes, and recordings of single recombinant $GABA_A$ receptor channels (Majewska and Schwartz, 1987; Majewska, 1990; Majewska et al., 1990; Majewska, 1992; Roberts, 1995; Akk et al., 2001). This action of PS occurs at a site on the $GABA_A$ receptor that is distinct from the site at which pregnane steroids act as positive allosteric modulators (Zaman et al., 1992; Park-Chung et al., 1997, 1999). In addition, PS potentiates NMDA receptor responses through an allosteric mechanism in which the channel opening frequency and duration are enhanced (Bowlby, 1993; Irwin et al., 1992, 1994; Fahey et al., 1995; Mukai et al., 2000). Recently, it has been demonstrated that PS also exerts a presynaptic action to enhance glutamate release and that this occurs optimally in a frequency-dependent fashion such that epileptic bursting might be selectively promoted (Meyer et al., 2002; Partridge and Valenzuela, 2002). These various actions of PS likely underlie its proconvulsant activity in vivo. However, in the hippocampal slice, PS did not induce epileptiform bursting by itself and did not dramatically alter the frequency of bursting induced by PTX and 4-AP. There was only a small enhancement in the frequency of PTX-induced bursting at early time points and no facilitation of 4-AP positive potentials. There was, however, a marked facilitation in the frequency of 4-AP-induced negative potentials. It is not apparent why PS does not induce epileptiform bursting in a similar fashion to PTX, since the block of $GABA_A$ receptor channels by PS is comparable to that of PTX (Mienville and Vicini, 1989), although the two antagonists do not appear to act at the same site (Shen et al., 1999). One possibility is that while PS can produce nearly complete block of steady-state $GABA_A$ receptor currents evoked by long applications of GABA (Shen et al., 1999), it has relatively less effect on rapid responses induced by synaptic activation (Shen et al., 2000). Since it is just such rapid, synaptically generated GABA responses that are relevant to the actions of PS on hippocampal circuit activity, this could explain why PS may not be as potent at inducing and modifying epileptiform activity as expected. In addition, as a charged molecule, PS may be less effective in penetrating the brain slice so that effective concentrations are reduced. This contrasts with the highly lipophilic pregnane steroids 5α,3α-P and 5β,3α-P which would easily access their receptor targets in the slice. Another factor that could explain the modest effect of PS on burst firing induced by the convulsant drugs is that bursting may already be at a maximal frequency as demonstrated by the experiments of Fig. 2 where increasing the 4-AP concentration from 55 to 75 μM only modestly and non-significantly increased the discharge rate. On the other hand, synchronous bursting of interneurons may not be maximal and this could explain why PS is able to dramatically facilitate the frequency of negative potentials.

In conclusion, these studies demonstrate that convulsant-induced bursting in the hippocampal slice, an in vitro epilepsy model, is sensitive to the anticonvulsant $GABA_A$ receptor modulating neurosteroid 5α,3α-P. The steroid suppressed epileptiform activity induced by the $GABA_A$ receptor antagonist PTX and

also by 4-AP, even though seizures induced by 4-AP are generally not sensitive to GABA modulating drugs in in vivo models. The slice model may be useful for evaluating the anticonvulsant potential of neuroactive steroids under conditions that avoid confounding factors such as differences in metabolism and brain penetration. In addition, in vitro slice models may be useful for defining the cellular actions that account for the anticonvulsant properties of these steroids.

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